IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Conf. No. 5768

Mohamed A. MARAHIEL et al. Group Art Unit: 1651

Application No.: 10/566,432 Examiner: Fernandez, S. E.

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For: METHOD FOR THE PRODUCTION OF CYCLIC MOLECULES

SUBMISSION OF TRANSLATION OF PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Further to Applicants' Amendment filing of April 29, 2011, Applicants submit herewith a translation of Applicants' priority document along with the verified statement as to the accuracy of the translation.

Applicants further submit that the priority document supports the claims as they currently stand. This means that Applicants are permitted to rely on the filing date of the priority document and the rejections based on prior art published after the filing date of the priority application, i.e., the Grunewald and Sieber references, are no longer valid.

Entry of this filing is respectfully requested and, based on the response filed on April 29, 2011 and this filing, an allowance of the application is requested.

No fee is believed to be due in connection with this filing.

However, please charge any fee deficiency or credit any overpayment to Deposit Account No. 50-1088.

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Date: June 30, 2011

United States Patent & Trade Mark Office

IN THE MATTER OF United States Patent Application No. 10/566,432

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"Method for the production of cyclic molecules"

I, Mrs Astrid Terry, a Technical Translator, of 11, Bounds Oak Way, Tunbridge Wells, Kent TN4 0UB do hereby declare that I am familiar with the English and German languages and that the attached English text is a true and correct translation into the English language of the priority document of the above application namely German Patent Application No. 10335584.7 in German.

Dated this 15th day of June 2011

Signed........Astrid Terry

THE FEDERAL REPUBLIC OF GERMANY

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The attached pages are a true and exact representation of the original documents filed as a Patent Application.

Munich, 16th September 2004
The German Patent and Trademark Office
On behalf of the President

Signed

Ebert

Patent Application

Method for the production of cyclic molecules

In the search for new pharmaceuticals, natural products are increasingly the focus of science and serve the latter as a lead structure for the development of new agents. Bacteria or fungi synthesize these pharmacologically relevant molecules, and their spectrum of activity extends from

- antibiotic (infectious diseases) to
- cytostatic (cancer) up to
- immunosuppressive (organ transplantation)

characteristics.

Within nature, the synthesis of these small molecules mostly occurs in large multienzymes which primarily produce peptides, polyketides or a hybrid of both.

Prominent examples for such compounds are penicillin, cephalosporin, daptomycin, epothilone, cyclosporine, a part of which has been successfully used in medicine for a long time. A common characteristic of these compounds is the cyclic structure which is decisive for the biological activity. Many of the aforementioned compounds

feature no or considerably reduced effectivity if they are present in linear form. In contrast to linear molecules, cyclic molecules have reduced conformation flexibility (free movement and rotation) due to the ring formation, which allows only the biologically active form to appear. In this context, nature has selected an interesting strategy which ensures that the synthesized molecule exists in only one modification and thus interacts specifically with only one "target" (attack destination) within the biological system. Targets are most frequently essential parts or functions of a cell, which are important for its survival, such as e.g. the cell wall or protein synthesis. As these molecules selectively eliminate bacterial, fungal or carcinogenic (cancer) cells or viruses while simultaneously protecting the body's own cell tissue, they are of enormous importance for the therapy of infectious diseases and cancer. In addition to that, they can also suppress the immune defense caused by T -cells, which effectively inhibits organ rejection with transplantations (cyclosporine).

Due to the intensive application in medicine many of these compounds have unfortunately lost their effectivity, as the systems to be fought have developed

resistance mechanisms. Furthermore, many potent agents possess very strong side effects, due to which their medical application is limited (e.g. nephrotoxicity of bacitracin). Hence, a big demand exists for new or optimized chemotherapeutics (antibiotics, cytostatics, immunosuppressants), which should feature as few side effects as possible and interact in a highly specific manner with their "target".

For the identification of such new agents, the potent cyclic natural products which are already known can serve as a lead structure and be systematically modified and tested for improved effectivity.

Such natural products are produced in the biological system by non -ribosomal peptide synthetases (NRPS) and cyclized by so called thioesterases/cyclases, which can be recombinantly overproduced in vitro with a good yield. These enzymes can reliably and efficiently transfer linear peptides of a given lead structure into cyclic molecules. In natural systems, the activation of the C -terminus (i.e. of the free carboxylic acid of the linear peptide) by a thioester leaving group - the cofactor phosphopantetheine - is the driving force of the cyclization reaction. In the artificial system, the

recombinant cyclase reacts with an abridged thioester -mimic of this natural cofactor (N -acetylcysteamie, SNAC). Abridged thioester -mimics are understood to be substances which

- imitate the function of the natural cofactor, however, are not of a natural origin,
- possess a thio leaving group and
- whose aliphatic chain is shorter than those of the natural cofactor phosphopantetheine.

DIAGRAM

Tyrocidine cyclase and surfactin cyclase have been characterized so far with the help of the SNAC leaving group. Many other biologically relevant cyclic compounds, such as e.g. fengycin, mycosubtilin, syringomycin and bacitracin do not show any cyclization activity with the respective enzyme on using the SNAC leaving group, which can be explained by an incorrect folding of the enzyme. Other compounds, such as e.g. CDA (calcium dependent antibiotic) and bacillibactin show in part a very bad conversion with the known substrate analoga.

The object of the present invention are non -natural, synthetic cofactors whose chemical qualities as leaving groups ensure an efficient enzyme acylation.

In contrast to the widely held belief among those skilled in the art, no "recognition" of the natural cofactor pantetheine by the enzyme takes place, thus the chemical transfer potential of the acyl residue to the active center within the enzyme is exclusively the decisive factor. The belief prevalent among those skilled in the art that the "recognition" of the natural cofactor pantetheine by the enzyme is the decisive factor for the cyclisation reaction, is presented in, for example, J W Trauger, R M Kohli, H D Mootz, M A Marahiel and C T Walsh, Nature 2000, 407: 215 -218; R Aggarwal, P Caffrey, P F Leadly, C J Smith and J Staunton, Journal of the Chemical Society Communications 1995, 15: 1519 -1520 and R S Gokhale, D Hunziker, D E Cane and C Khosla, Chemical Biology 1999, 6: 117 -125.

In contrast to the established SNAC substrates, thiophenol, e.g., features, as a charge -stabilized leaving group according to the present invention, no structural analogy at all to the natural cofactor, provides, however, a significantly better leaving group

quality, as the thiol is in conjugation with an aromatic benzene ring. Within other leaving groups according to the present invention the thiol function or the hydroxy function is bound to an sp^3 C atom, which is directly bound to the aromatic ring (α -C atom), in such a way that the aromatic system has an inductive effect on the thio groups or the hydroxy groups. Such leaving groups according to the present invention are referred to in the following as araliphatic thio leaving groups or araliphatic hydroxy leaving groups. The expert skilled in the art knows that the inductive effect of an aromatic system has a stabilizing effect on the groups bound to an α -C -Atom, thus, increasing their leaving group quality. This can be read about e.g. in Michael B. Smith & Jerry March: March's Advanced Organic Chemistry. Reactions, Mechanisms, and Structure. 5th Edition 2000, John Wiley & Sons Inc., New York/Chichester/Brisbane/Toronto/Singapore. In the case of the SNAC, neither a conjugation with an aromatic or heteroaromatic system nor stabilization by the inductive effect of an aromatic system in an α -position to the carbon atom, to which the thio group is bound, is available, thus, many enzymes do not show any activity with these substrates or feature low k_{cat}/K_M values.

DESCRIPTION AND STATE OF THE ART

Many valuable pharmaceuticals feature cyclic structures, wherein the rings of these cyclic structures are composed of 5 or more atoms. Methods of synthetic chemistry for manufacturing cyclic compounds known in the state of the art feature numerous disadvantages. These disadvantages include, for example, but not exclusively, low yields of the cyclic products, the necessity of protective groups to block or to protect reactive functional groups, as well as the need to carry out these reactions in organic solvents. These synthetic problems can be overcome by enzymatic methods. EP 0 832 096 B1 describes a method in which a non -oxidized N -terminal cysteine of a first oligopeptide is reacted with the C -terminal thioester of a second oligopeptide. The reaction is catalyzed by a thiol, wherein the thio group is bound directly to an aromatic or heteroaromatic ring. In this, a β -amino thioester is formed as an intermediate, followed by spontaneous intramolecular rearrangement, wherein the amide bond of the ligation product of the two oligopeptides is formed. Disadvantages of this method are that the first oligopeptide must possess an N -terminal cysteine and that it is not able to undergo cyclization reactions.

U.S. 6,307,018 B1, in contrast, describes a general method of binding a first C -terminal α -thioester peptide with a second N -terminal amino acid peptide segment, in which the N -terminal amino acid peptide segment does not need to possess an N -terminal cysteine. The second oligopeptide, however, must possess a secondary amino group, which is bound by the N atom of this secondary amino group to a non -oxidized sulfhydryl group of an aromatic thiol. The aromatic thiol can be either a thiophenol, benzylmercaptane, or an S -alkyl benzylmercaptane. Another disadvantage of U.S. 6,307,018 B1 is that either the C -terminus of the first or the N -terminus of the second oligopeptide must be glycine. The method is not suitable for the cyclization of peptides.

US 2002/0192773 A1 describes a method for the enzymatic production of macrocyclic molecules, in which recombinant thioesterase domains (TE domains, cyclases) derived from a PKS or NRPS multidomain system are reacted with a substrate, wherein the substrate contains an acyl residue which is activated by a thioester leaving group and an adjacent nucleophile. The activated acyl residue and nucleophile are separated from one another by a linear

backbone. Hereby, a disadvantage is that the leaving group is not charge -stabilized.

Due to insufficient cyclization activity of many enzymes on using leaving groups which are structurally analogous to the natural cofactor, such as, for example, coenzyme A, phosphopantetheine and N -acetylcysteamine TE -domains are considerably limited in their application, however. The present invention overcomes this limitation by the use of novel leaving groups and now enables the development of diverse libraries of cyclic active agents of many pharmacologically significant molecule classes.

Surprisingly, and in contradiction with the technical state of the art, it was found that the recognition of the substrates by the enzymes plays no role whatsoever in the cyclization of peptides and proteins and that charge -stabilized thio and hydroxy compounds represent good leaving groups for the acylation reaction of peptide cyclases. Charge -stabilized thio - and hydroxy compounds are thereby understood to be aromatic or heteroaromatic ring systems, wherein a hydroxy or thio group is bound to one of the ring atoms or to a carbon atom which is bound to the ring system.

The invention at hand provides substrates with whose help enzymatic cyclization of such peptides and proteins which were not accessible to cyclization according to the state of the art is possible. Moreover, the yield of proteins and peptides, which can be cyclized with methods available according to the state of the art, can be increased with the help of the substrates according to the present invention. Furthermore, the invention at hand provides a method to chemically modify further substrates engaged in the cyclization of peptides and proteins and thereby makes them more easily accessible for cyclization.

AIM OF THE INVENTION

It is the aim of the present invention to improve the method for the production of cyclic peptides by the reaction of linear peptides with peptide cyclases, wherein "improvement" means an increased yield of the cyclic peptide and/or acceleration of the cyclization reaction and/or cyclization of peptides which can not be cyclized with methods used thus far. This aim is achieved, according to the present invention, by the provision of a method by means of substrates in the form of linear peptides wherein there linear peptide have

charge -stabilized leaving groups and selectively acylate the center of peptide cyclases. "Substrates" are understood here to be linear peptides on which a nucleophilic charge -stabilized leaving group according to the present invention is chemically bound. In this, charge -stabilized thio and hydroxy compounds are understood to be aromatic or heteroaromatic ring systems in which a hydroxy or thio group is bound to one of the ring atoms or on a carbon atom which is bound to the ring system, wherein the chemical structure of the aromatic or heteroaromatic system is chosen in such a way that a negative charge occurring on the thio or hydroxy group is stabilized. The method according to the present invention leads to higher yields of cyclic peptides and/or increases its yields and, for the first time, allows peptides such as fengycin, mycosubtilin, syringomycin and bacitracin to cyclize as well, which are not able to be cyclized with the methods according to the state of the art.

The provision of the substrates according to the present invention is carried out via the synthesis of the linear peptide with help from the standard methods of solid phase peptide synthesis known to persons skilled in the art, with subsequent coupling of the free carboxylic acid

of the linear peptide (the free peptide acid) to the thiol or hydroxy leaving group according to the present invention, optional purification of the substrate obtained in this way according to the present invention, with subsequent reaction of the substrate obtained in this way according to the present invention with a peptide cyclase and purification of the cyclic peptides obtained in this way.

For this, 1 equivalent (eq) of the free peptide acid is reacted with 2 eq dicyclohexylcarbodiimide (DCC), 2 eq N -hydroxybenzotriazole (HOBt) and 10 eq of the respective leaving group and stirred for 30 min in THF. After addition of 0.5 eq potassium carbonate, the reaction is agitated for a further 2.5 h and then filtrated to remove precipitated dicyclohexylurea (DCU). The solvent is removed using the rotary evaporator and the peptide is deprotected with 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisopropylsilane for 3 h. The reaction mixture is then added to ice -cold diethyl ether, subsequent to which the substrate precipitates. This step represents purification, by which reaction byproducts are removed and leads to a substrate purity of up to 80%, which is generally satisfactory for a further reaction of the substrate with a peptide cyclase. Optionally, the

purity of the substrate can be subsequently increased by means of preparative HPLC. If the linear peptide next to the C -terminal free COOH group has further free COOH groups within the peptide chain, such as e.g. COOH groups from glutamic acid and/or aspartic acid, then these non C -terminal free COOH groups must be protected before the reaction of the linear peptide with an activating reagent with a suitable orthogonal protective group, which must then be cleaved off after production of the substrate according to the present invention. Suitable protective groups and suitable methods of their removal are known to persons skilled in the art and can be read about, for example, in Theodora W. Greene and Peter G. M. Wuts, "Protective groups in organic synthesis," 2.sup.nd Edition 1991, John Wiley & Sons Inc., New York/Chichester/Brisbane/Toronto/Singapore.

The purified substrate with the leaving group according to the present invention is then incubated with the respective peptide cyclase in the ratio of 1 (enzyme): 100 (substrate) in 25 mM HEPES, 50 mM NaCl at pH 7 and room temperature for 30 -60 minutes. The production of the HEPES solution is known to persons skilled in the art and was described in J. Sambrook, E. F. Fritsch and T. Maniatis: Molecular Cloning: A Laboratory Manual Vol. I

-III, Cold Spring Harbor Laboratory Press, 1982. The identification and quantification of the reaction product is carried out by means of analytical HPLC.

As an alternative to the activation reagent DCC, the substrates according to the present invention can also be reacted by reaction of the peptide acid with the respective leaving group in the presence of other reagents activating the C -terminus of the peptide acid. Equivalents are known and can be used without leaving the area protected by the patent claims. Hereby, the activation reagents known to persons skilled in the art include, for example, DCI, PyClop, HBTU, HATU, HOSu, TBTU, T3P, BopCl and 3 -Cl -1 -pyridiniumiodide. Apart from HOBt listed above, the substances HOAt and HONB, which are known to persons skilled in the art, can also be used, for example, as coupling additives. It is known to persons skilled in the art that these reactions are effectively carried out with the addition of a base, such as e.g. DIPEA. Furthermore, different solvents for use in the methods mentioned are known to persons skilled in the art. Skilled persons can produce these combinations of activation reagents, coupling additives, bases, and solvents themselves with their general knowledge and standard literature.

Charge -stabilized leaving groups are understood in the present invention to be chemical compounds which possess a thio or hydroxyl group and in which the free electron pair of the thiolate or hydroxylate ion released by the acylation reaction stands in conjugation with other electron pairs from, for example, but not exclusively, C=C or C=N double bonds or in which the thio or hydroxy group is bound to a carbon atom which is, for its part, bound to an aromatic or heteroaromatic ring. Such compounds are, e.g. oxo - and thio -aromatic, and oxo - and thio -heteroaromatic compounds, but also charge -stabilized aliphatic oxo and thio leaving groups. These leaving groups, such as e.g. thiophenol, phenol, 2,3,4,5,6 -pentafluorophenol, mercaptoanisoles and thiocresols as well as 2 -hydroxypyridine and 2 -thiopyridine work in the acylation reaction of peptide cyclases which possess no similarity with the natural cofactor at all, and feature improved characteristics for in vitro cyclization reactions.

This is to be explained in the following, for example, but not exhaustively, with the example of thiophenol:

The thiophenol leaving group features, apart from the thiol function, no structural similarity with natural 4' -phosphopantetheine cofactor. The thiol function is directly bound to an aromatic phenyl ring. This structural characteristic causes a higher reactivity of this compound in relation to the leaving groups already described. During nucleophilic attack of the activated Ser (=serine) of the catalytic triad in the active center of the enzyme, this leaving group is released as a thiophenolate ion. The resulting negative charge at the sulfur atom can, in this, be delocalized by the adjacent phenyl ring very well.

DIAGRAM

An increase and stabilization of the electron density of this kind does not occur with SNAC, CoA and Ppant leaving groups. In these cases, the negative charge remains localized at the sulfur atom. Since as a rule, however, the quality of a leaving group is proportional to its chemical stabilization, SNAC, CoA, and Ppant are worse leaving groups than thiophenol from a chemical viewpoint.

It is known to persons skilled in the art that the leaving ability and, therefore, the quality of a leaving

group is dependent upon the ability of the leaving group to stabilize a negative charge. Stabilization of a charge is understood here by skilled persons to be the distribution of charges or partial charges over several atoms or bonds, so that this charge or partial charge is not localized at a unique atom or bond within a molecule. In this, two different possibilities for charge stabilization of organic molecules, which are generally called mesomeric or resonance effects (M effects) and inductive effects (I effects), are known to persons skilled in the art. Persons skilled in the art understand a mesomeric or resonance effect to be the quick and reversible moving around of π electron pairs, which occurs in systems which possess conjugated π bonds. It is known to persons skilled in the art that the mesomeric effect is effective over large distances and, therefore, on many bonds when a corresponding extended conjugated π system exists. In ring compounds with conjugated π systems, substituents also take part in the mesomerism, as long as they have free π electron pairs at their disposal or can absorb these. If a charge is to be stabilized in a substituted ring compound with a conjugated π system and substituents with mesomeristic capacities, then it depends on the position of the substituents to one another if and which of these

substituents in fact take part in charge -stabilization by mesomerism. This is known to persons skilled in the art.

If an atom possesses a higher electronegativity and, therefore, a stronger attraction to the binding electrons than its neighboring atom which is bound to it by a σ bond, or if an atom is bound with further atoms or atom groups which have an electron withdrawing effect, the electron cloud of the σ bond mentioned here will be moved in the direction of electron withdrawal, i.e. polarized. This polarization of a σ bond is described as a partial charge, since it concerns a slight movement of electron clouds here and this movement does not lead to the occurrence of integer multiples of the elementary charge at a certain atom. The polarization of σ bonds caused by different electronegativities and/or different electron withdrawal of atoms and atom groups is also described as an inductive effect by persons skilled in the art. That the inductive effect is the biggest for neighboring bonds and decreases quickly with increasing distance to the atom or atom group which causes it is known to persons skilled in the art. This can be read about, e.g. in Michael B. Smith & Jerry March: March's Advanced Organic Chemistry. Reactions, Mechanisms, and Structure. 5th

Edition 2000, John Wiley & Sons Inc., New York/Chichester/Brisbane/Toronto/Singapore.

Persons skilled in the art differentiate between positive and negative mesomeric or inductive effects, respectively. Such an effect is described as positive when it increases the electron density in the form of a charge or partial charge on an atom or atom group (+M effect, +I effect), negative when it decreases the electron density (-M effect, -I effect). If several substituents are located, for example, on an aromatic system, they exert their M effects and I effects independently of one another and can have an intensifying, but also an opposing effect amongst each other in relation to charge stabilization on a certain atom. As a rule, mesomeric effects are stronger than inductive. Therefore, in the invention at hand, such charge -stabilized leaving groups are preferably chosen, in which a hydroxy or thio group is bound to one of the ring atoms of an aromatic, heteroaromatic or araliphatic system or to a carbon atom which is bound to the ring system, wherein the chemical structure of the aromatic, heteroaromatic or araliphatic system is so chosen that the sum of the mesomeric and inductive effects of the functional groups contained exerts an electron withdrawal

on the thiolate or hydroxylate ion and thus stabilizes the negative charge thereof.

A further important criterium for the quantification of the leaving group quality is the pK_A value of a chemical compound: the higher the pK_A value, the worse the respective leaving group is. CoA, Ppant and SNAC have pK^A values of 10 -11, while thiophenol features a pK_A value of 8. From that, it can be said that thiophenol can overcompensate for its lacking structural consistency with the natural phosphopantetheine cofactor surprisingly and contrary to the state of the art by its high chemical reactivity, which is also true for other aromatic, heteroaromatic and charge -stabilized araliphatic thiol or hydroxyl compounds. In this, such charge -stabilized aromatic, heteroaromatic and araliphatic thiol and hydroxyl compounds whose pK_A value is smaller or equal to 10, preferably smaller or equal to 8, are used advantageously as leaving groups. The ring systems of the aromatic, heteroaromatic and araliphatic thiol and hydroxy compounds according to the present invention can be substituted by one or more substituents with positive or negative inductive or mesomeric effects, wherein the totality of the effects of all the substituents at hand causes an electron withdrawing and thus charge

-stabilizing force on the thiolation or hydroxylation released during the enzymatic cyclization.

In the use of charge -stabilized thiol and hydroxy compounds, such enzymes also show cyclization activity which were classified as inactive with the use of the leaving groups known so far (approx. 2/3 of all examined so far). Enzymes which also cyclize during use of SNAC as a leaving group show better kinetic properties with k_{cat}/K_M values increased up to 15 times with constant regioselectivity and stereoselectivity, when thiophenol derivatives are used in place of SNAC leaving groups. This was demonstrated with the example of surfactin thiophenol (see FIG. 4). Surfactin likewise shows improved reaction rates during cyclization when o -mercapto anisole, m -mercapto anisole, or p -mercapto anisole or o -thiocresole, m -thiocresole, or p -thiocresole are used as leaving group.

The catalysis by peptide cyclases can be broken down into two partial steps:

The first partial step is the formation of the peptidyl -O -TE -intermediate through the acylation of the activated Ser residue of the catalytic triad.

The second partial step consists of the deacylation of the Ser residue by a functional group of the bound peptide chain as an internal nucleophile.

Thioester -bound leaving groups can exclusively influence the catalytic efficiency of the first partial step: the formation of the peptidyl -O -TE intermediate. Experiments with the new leaving group thiophenol confirm this (see FIG. 4 to FIG. 6). A mutation within the active center of the enzyme shows no activity which confirms the acylation by the leaving group and the following enzymatic cyclization.

The following aromatic, heteroaromatic, and araliphatic basic elements serve as charge -stabilized leaving groups:

DIAGRAM

with

A=O, S and

as well as R1, R2, R3, R4 and R5, which are independent of one another:

$-NO_2$, $-CN$, $-F$, $-Cl$, $-Br$, $-I$, $-CH_2Cl$, $-SO_3H$, $-H$, $-NH_3^+$, $-NL_3^+$, $-C(=O)L$, $-C(=O)Het$, $-O^-$, $-NL_2$, $-NH_2$, $-OL$, $-OH$, $-NHC(=O)L$, $-OC(=O)L$, $-SL$, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl for a corresponding aromatic residue in which up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus, wherein the conditions are chosen in such a way that, in temperatures lower than 200° C and atmospheric pressure, no explosive substances are formed and the compounds comprised of linear peptides, and the

leaving groups according to the present invention bound to those, are not hydrolytically cleaved in these conditions,

DIAGRAM

with

A=O, S and

as well as R1 and R2, which may be independent of one another:

$-NO_2$, $-CN$, $-F$, $-Cl$, $-Br$, $-I$, $-CH_2Cl$, $-SO_3H$, $-H$, $-NH_3^+$, NL_3^+, $-C(=O)L$, $-C(=O)Het$, $-O^-$, $-NL_2$, $-NH_2$, $-OL$, $-OH$, $-NHC(=O)L$, $-OC(=O)L$, $-SL$, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon

atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus, wherein the conditions are chosen in such a way that, in temperatures lower than 200° C and atmospheric pressure, no explosive substances are formed and the compounds comprised of linear peptides and the leaving groups according to the present invention bound to those are not hydrolytically cleaved in these conditions, whereby it is known to persons skilled in the art, that substituents bound to C -4 or C -6 of the pyridine ring do not cause a charge stabilization of the hydroxy or thiol substituent that is bound to C -2,

DIAGRAM

with

A=O, S, and

Z=O, S,

as well as R1, R2, and R3, which may be independent of one another:

$-NO_2$, $-CN$, $-F$, $-Cl$, $-Br$, $-I$, $-CH_2Cl$, $-SO_3H$, $-H$, $-NH_3^+$, $-NL_3^+$, -C(=O)L, - -C(.dbd.O)Het, - -O.sup. -, - -NL.sub.2, - -NH.sub.2, - -OL, - -OH, - -NHC(.dbd.O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl my be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus, wherein the conditions are chosen in such a way that, in temperatures lower than 200° C and atmospheric pressure, no explosive substances are formed

and the compounds comprised of linear peptides and the leaving groups according to the present invention bound to those are not hydrolytically cleaved in these conditions,

DIAGRAM

with

A=O, S, and

Z=O, S,

as well as R1, R2, and R3, which may be independent of one another:

$-NO_2$, $-CN$, $-F$, $-Cl$, $-Br$, $-I$, $-CH_2Cl$, $-SO_3H$, $-H$, - $-NH_3^+$, $-NL_3^+$, $-C(=O)L$, $-C(=O)Het$, $-O^-$, $-NL_2$, $-NH_2$, $-OL$, $-OH$, $-NHC(=O)L$, $-OC(=O)L$, $-SL$, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched;

-cycloalkyl and -cycloalkenyl stand for a group with 3

to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus, wherein the conditions are chosen in such a way that, in temperatures lower than 200° C and atmospheric pressure, no explosive substances are formed and the compounds comprised of linear peptides and the leaving groups according to the present invention bound to those are not hydrolytically cleaved in these conditions,

DIAGRAM

with

A=O, S and as well as R1, R2, R3, R4 and R5, which are independent of one another:

$-NO_2$, $-CN$, $-F$, $-Cl$, $-Br$, $-I$, $-CH_2Cl$, $-SO_3H$, $-H$, $-NH_3^+$, $-NL_3^+$, $-C(=O)L$, $-C(=O)Het$, $-O^-$, $-NL_2$, $-NH_2$, $-OL$, $-OH$, $-$ $-HC(=O)L$, $-OC(=O)L$, $-SL$, $-CO_2^-$, -alkyl,

-alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus, wherein the conditions are chosen in such a way that, in temperatures lower than 200° C and atmospheric pressure, no explosive substances are formed and the compounds comprised of linear peptides and the leaving groups according to the present invention bound to those are not hydrolytically cleaved in these conditions.

Furthermore, these leaving groups can also replace the natural cofactor for other artificial reactions with enzymes of the non -ribosomal peptide synthetases in vitro. Such a reaction is represented by the condensation reaction to form a peptide bond, catalyzed by the condensation domain (C domain) which also operates with thioester -bound substrates.

Surprisingly, and in contradiction with the technical state of the art, it was found that the recognition of the substrates by the respective enzyme plays no role whatsoever. Thus, the invention at hand provides a new and, for the average person skilled in the art, surprising further development of the method described in US 2002/0192773 A1 for the enzymatic production of macrocyclic molecules, in which purified, isolated thioesterase domains (TE domains, cyclases) derived from a PKS or NRPS multidomain system are reacted with a substrate.

The substrates in question include linear peptides and lipopeptides with 5 to 22 monomeric units, such as e.g. amino acids. Substrates are, for example, fengycin, mycosubtilin, bacillibactin, CDA, surfactin, bacitracin

or syringomycin and further substrates which are already described in US 2002/0192773 A1, as well as prystinamycin, whereby the substrates indicated additionally feature a leaving group according to the present invention. Several of these substrates are depicted in the following:

DIAGRAM

Bioactive Peptides

DIAGRAM

Structure of a fengycin -thiophenol substrate

DIAGRAM

Structure of a surfactin -thiophenol substrate

The method according to the present invention also provides, in comparison with the state of the art, an improvement for such linear peptides which could already be cyclized by methods known to persons skilled in the art, since the method according to the present invention

accelerates the reaction rate of the cyclization and/or leads to higher yields of the cyclic peptides.

The enzymes in question include purified, isolated thioesterase domains or peptide cyclases from NRPS or PKS systems, such as e.g. the corresponding domains or cyclases of fengycin, mycosubtilin, bacillibactin, CDA, surfactin, bacitracin, syringomycin, tyrocidin, prystinamycin and all other peptide cyclases, thioesterases and purified, isolated thioesterases listed in US 2002/0192773 A1.

The linear peptide contains proteinogenic and non -proteinogenic amino acids in its backbone. Residues and/or functional groups, which do not derive from amino acids, can also be embedded in this backbone, such as e.g. saturated or unsaturated carbon spacers. The residues and/or functional groups facultatively embedded in the backbone were already described in US 2002/0192773 A1. For this, the leaving group according to the present invention is attached either to the C -terminal carboxylic acid group or to a side chain carboxylic acid.

The leaving group technology according to the present invention can be used for the production of substance

libraries for cyclic peptides and proteins, producing new substrate variants of structurally important molecules (for example, fengycin, mycosubtilin, syringomycin, CDA, etc.), which have so far shown no or little activity with the usual leaving group SNAC, and testing them for improved biological properties (antibiotic, antiviral, antifungal, cytostatic). The substrate variants are produced by combinatorial solid phase peptide synthesis and provided with the new leaving groups according to the aforementioned, general instruction. In this, a substance library for peptide antibiotics adapted to target cells is preferably produced, whereby cyclic peptide antibiotics which were produced with the help of the method according to the present invention are meant.

The method according to the present invention can be used for the production of cyclization kits which provide means for the coupling of charge -stabilized leaving groups according to the present invention as well as peptide cyclases, so that linear peptides can be reacted with the leaving groups made available, at first to form substrates according to the present invention and subsequently with the peptide cyclases made available, to form cyclic peptides. The producer of the kits according to the present invention knows from general knowledge how

to produce, formulate and store the single components of the kit, e.g. buffers.

The cyclic peptides and proteins produced by the method according to the present invention can be used as pharmaceuticals for patients for the therapy, diagnosis and prophylaxis of diseases in which bacterial and/or viral infections arise. Furthermore, the cyclic peptides and proteins according to the present invention can be used as pharmaceuticals for patients for the therapy, diagnosis and prophylaxis of tumor diseases as well as in transplantation medicine, provided that they feature cytostatic and/or immunosuppressive properties. The term patient refers equally to humans and vertebrates. Thus, the pharmaceuticals can be used both in human and veterinary medicine. Pharmaceutically acceptable compositions of compounds according to the claims can be available as dimers up to oligomers or as salts, esters, amides, or "prodrugs" thereof, provided that, according to reliable medical evaluation, they do not cause excessive toxicity, irritations or allergic reactions to patients. The therapeutically effective compounds according to the present invention can be administered to patients as part of a pharmaceutically acceptable composition either in oral, rectal, parenteral,

intravenous, intramuscular, subcutaneous, intracisternal, intravaginal, intraperitoneal, intravascular, intrathecal, intravesical, topical, local form (powder, salves or drops) or in aerosol form, wherein the intravenous, subcutaneous, intraperitoneal or intrathecal administration can be carried out continuously by means of a pump or dosage unit. Forms of dosage for local administration of the compounds according to the present invention include salves, powders, suppositories, sprays and inhalants. Hereby, the active component is mixed under sterile conditions with a physiologically acceptable carrier and possible preservatives, buffers, diluents and propellants according to need.

EMBODIMENTS

Embodiment 1: Production and purification of the fengycin -thiophenol substrate as well as cyclization

The linear fengycin substrate is first produced according to standard methods of peptide solid phase synthesis. The peptide sequence is: Acetyl -Glu -D -Orn -Tyr -D -Thr -Glu -D -Ala -Pro -Gln -D -Tyr -Ile -COOH. In the next step, 0.1 mMol DCC, 0.1 mMol HOBt and 0.5 mMol of thiophenol are added to 0.05 mMol of the peptide and

dissolved in 2 ml THF. The mixture is stirred for 30 min at RT, and 0.05 mMol of potassium carbonate is added. The mixture is stirred for a further 2.5 h at RT, subsequently solid DCH is removed by filtration and the solvent is evaporated using the rotary evaporator. The deprotection of the peptide side chains is carried out for 3 h in 2 ml of 95% TFA, 2.5% water and 2.5% triisopropylsilane. The mixture is then poured into 50 ml of ice -cold diethyl ether and the resulting solid is separated by centrifugation. The purification of the solid is carried out by means of preparative HPLC with a Nucleodur C_{18} column (pore size 100 Å, particle size 7 µM, diameter 10 mm, length 250 mm, Macherey -Nagel) with a gradient of 10% acetonitrile in water/0.1% TFA up to 70% acetonitrile in water/0.1% TFA in 40 min at a flow rate of 6 ml/min. The retention time of the cyclized fengycin (see Fig. 1) is 19 min. The yield is between 70 and 80%.

The products are tested for purity and identity with LC -MS and MALDI -TOF mass spectrometry.

Embodiment 2: Production and purification of the fengycin -benzylmercaptane substrate as well as cyclization

Production and purification of the fengycin -benzylmercaptane substrate were carried out analogously to embodiment 1, wherein in embodiment 2 0.05 mMol benzylmercaptane is used instead of 0.05 mMol thiophenol. The yield of the cyclized fengycin is approx. 70%.

Embodiment 3: Production and purification of further fengycin substrates as well as cyclization

Fengycin is reacted with further leaving groups according to the present invention as described in embodiments 1 and 2. These are 2 -mercaptopyridine, p -nitrothiophenol and pentafluorothiophenol. The cyclization of these fengycin substrates yields significantly higher percentages of the not enzymatically catalyzed cyclization product or the hydrolyzed product than in the case of using thiophenol or benzylmercaptane, respectively.

Table 1

The linear peptides fengycin, surfactin, CDA and syringomycin are reacted with thiophenol as described in embodiment 1 and subsequently enzymatically cyclized. Tab. 1 shows the results of the mass spectrometric

measurement of the substances yielded according to the present invention.

TABLE

[List of reference numerals]

Fig. 1: HPLC of the reaction of fengycin -thiophenol with the fengycin -peptide cyclase

HPLC -MS with a reversed phase Nucleodur C_{18} column (Macherey and Nagel, 250/3, pore diameter: 100 Å, particle size: 3 µm) with the following gradient: 0 -35 min, 30 -60% acetonitrile/0.1% TFA in water/0.1% TFA; 0.4 mL/min, 40°C. 1 shows the control reaction with a mutated (inactivated) enzyme. 2 shows the incubation with the native enzyme (active). Su=substrate, Cy=cyclic product, Hy=hydrolyzed product.

Fig. 2: HPLC of the reaction of surfactin -thiophenol with the surfactin -peptide cyclase

HPLC -MS with a reversed phase Nucleodur C_{18} column (Macherey and Nagel, 250/3, pore diameter: 100 Å, particle size: 3 µm) with the following gradient: 0 -35

min, 30 -60% acetonitrile/0.1% TFA in water/0.1% TFA; 0.4 mL/min, 40.degree. C. 1 shows the control reaction without enzyme. 2 shows the incubation with the native enzyme (active). Su=substrate, Cy=cyclic product, Hy=hydrolyzed product, (Cy) non -enzymatically catalyzed cyclization by an amino side group within the peptide sequence on position 3 (Dap).

Fig. 3: Fengycin -peptide cyclase

5 µM of the recombinant fengycin -peptide cyclase, which showed no cyclization activity in previous experiments with conventional SNAC -substrates, is incubated with 100 µM fengycin -thiophenol for 10, 30, 40, 50, 60 min at room temperature in 25 mM HEPES, 50 mM NaCl at pH 7 in a total volume of 50 µL. With this measurement, the linear range for further kinetic studies is determined. The reactions are stopped by the addition of 35 µL TFA (4% in water) and examined by analytic HPLC with a Nucleodur C_{18} column (Macherey and Nagel, 250/3, pore diameter: 100 Å, particle size: 3 µm) with the following gradient: 0 -35 min, 30 -60% acetonitrile/0.1% TFA in water/0.1% TFA; 0.4 mL/min, 40° C. Kinetic examinations are carried out at different points in time at substrate concentrations of 50 µM up to 1000 µM and the kinetic parameters KM and

kcat are taken from the Lineweaver -Burk plot. For fengycin -thiophenol, a K_M of 461 µm and a k_{cat} of 0.33 min $^{-1}$ result from the cyclization reaction.

Fig. 4: Surfactin -peptide cyclase

In the case of surfactin -peptide cyclase, kinetic reference data with a SNAC -substrate exists. In the case of surfactin -thiophenol, a K_M of 126 µm and a k_{cat} of 5.6 min $^{-1}$ are determined for the cyclization reaction, which corresponds to a k_{cat}/K_M value of 0.04 µm $^{-1}$ min $^{-1}$. Compared with that the kinetic efficiency of surfactin -SNAC, represented by the k_{cat}/K_M value 0.0029 µm $^{-1}$ min $^{-1}$, is 14 times less than with surfactin -thiophenol.

Fig. 5: CDA peptide cyclase

A similar result is obtained for the cyclization of CDA -thiophenol with the "calcium dependent antibiotic" peptide cyclase (CDA). The K_M value for the thiophenol substrate is 10.7 µm, and the k_{cat} value amounts to 0.21 min $^{-1}$. The kinetic efficiency of the thiophenol substrate, with a k_{cat}/K_M value of 0.02 µm $^{-1}$min $^{-1}$ is 10 times larger than in comparison with the k_{cat}/K_M value of the SNAC substrate (k_{cat}/K_M=0.0021 µm $^{-1}$ min $^{-1}$).

Fig. 6: Syringomycin peptide cyclase

In the case of syringomycin peptide cyclase, no kinetic reference data with a SNAC substrate exists, as the SNAC substrate showed no activity in previous experiments. In the case of syringomycin -thiophenol, a K_M of 32.9 µM and a k_{cat} of 0.805 min $^{-1}$ are determined for the cyclization reaction, which corresponds to a k_{cat}/K_M value of 0.024 µM $^{-1}$min $^{-1}$.

Fig. 7: HPLC of the reaction of surfactin -2 -thiocresole with the surfactin peptide cyclase

HPLC -MS with a reversed phase Nucleodur C_{18} column (Macherey and Nagel, 250/3, pore diameter: 100 Å, particle size: 3 µm) with the following gradient: 0 -35 min, 30 -60% acetonitrile/0.1% TFA in water/0.1% TFA; 0.4 mL/min, 40°C. 1 shows the control reaction with a mutated (inactivated) enzyme. 2 shows the incubation with the native enzyme (active). Su = substrate, Cy = cyclic product, Hy = hydrolized product.

Fig. 8: HPLC of the reaction of surfactin -4 -methoxythiophenol with the surfactin peptide cyclase

HPLC -MS with a reversed phase Nucleodur C_{18} column (Macherey and Nagel, 250/3, pore diameter: 100 Å, particle size: 3 µm) with the following gradient: 0 -35 min, 30 -60% acetonitrile/0.1% TFA in water/0.1% TFA; 0.4 mL/min, 40°C. 1 shows the control reaction with a mutated (inactivated) enzyme. 2 shows the incubation with the native enzyme (active). Su = substrate, Cy = cyclic product, Hy = hydrolized product.

[Patent claims]

1. Method for the production of cyclic peptides, in which

- a peptide cyclase is brought in contact with a linear peptide,
- the linear peptide contains an acyl residue, which is activated by a nucleophilic leaving group bound chemically with this acyl residue,
- the activated acyl residue of the linear peptide selectively acylates the center of the peptide cyclase, wherein the nucleophilic leaving group is cleaved off during formation of the cyclic peptide and
- cyclic peptides with rings of at least 5 atoms are formed,

characterized in that

- the nucleophilic leaving group, which is chemically bound to the acyl residue of the linear peptide and which activates the latter, is charge -stabilized and
- the charge -stabilized leaving group is bound to the acyl group of the C -terminal carboxylic acid group of the peptide.

2. Method for the production of cyclic peptides according to claim 1, characterized in that the charge -stabilized leaving groups are aromatic, heteroaromatic or

araliphatic compounds, on which a hydroxy or thio group is bound to one of the ring atoms or to a carbon atom bound to the ring system.

3. Method for the production of cyclic peptides according to one of the claims 1 or 2, characterized in that the peptide cyclase is a NRPS or PKS cyclase, preferably a purified, isolated thioesterase domain.

4. Method for the production of cyclic peptides according to one of the claims 1 to 3, characterized in that the linear peptide contains proteinogenic and/or non -proteinogenic amino acids in its backbone, whereby residues which do not derive from amino acids can also be embedded in the backbone.

5. Method for the production of cyclic peptides according to one of the claims 1 to 4, characterized in that the charge -stabilized leaving group is a compound of the formula

DIAGRAM

wherein applies:

A=O, S

and whereby R1, R2, R3, R4 and R5 may be independent of one another:

$-NO_2$, -CN, -F, -Cl, -Br, -I, $-CH_2Cl$, $-SO_3H$, -H, $-NH_3^+$, $-NL_3^+$, -C(=O)L, -C(=O)Het, - -O $^-$, $-NL_2$, $-NH_2$, -OL, -OH, -NHC(=O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus.

6. Method for the production of cyclic peptides according to one of the claims 1 to 5, characterized in that the charge -stabilized leaving group is a compound of the formula

DIAGRAM

wherein applies:

A=O, S

and whereby R1 and R2 may be independent of one another:

$-NO_2$, -CN, -F, -Cl, -Br, -I, $-CH_2Cl$, $-SO_3H$, -H, $-NH_3^+$, $-NL_3^+$, -C(=O)L, -C(=O)Het, - $-O^-$, $-NL_2$, $-NH_2$, -OL, -OH, -NHC(=O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon

atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus.

7. Method for the production of cyclic peptides according to one of the claims 1 to 6, characterized in that the charge -stabilized leaving group is a compound of the formula

DIAGRAM

wherein applies:

A=O, S and

Z=O, S,

and whereby R1, R2, and R3 may be independent of one another:

$-NO_2$, -CN, -F, -Cl, -Br, -I, $-CH_2Cl$, $-SO_3H$, -H, $-NH_3^+$, $-NL_3^+$, -C(=O)L, -C(=O)Het, $-O^-$, $-NL_2$, $-NH_2$, -OL, -OH, -NHC(=O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus.

8. Method for the production of cyclic peptides according to one of the claims 1 to 7, characterized in that the charge -stabilized leaving group is a compound of the formula

DIAGRAM

wherein applies:

A=O, S and

Z=O, S,

and whereby R1, R2, and R3 may be independent of one another:

$-NO_2$, -CN, -F, -Cl, -Br, -I, $-CH_2Cl$, $-SO_3H$, -H, $-NH_3^+$, $-NL_3^+$, -C(=O)L, -C(=O)Het, $-O^-$, $-NL_2$, $-NH_2$, -OL, -OH, -NHC(=O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl, wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in

which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus.

9. Method for the production of cyclic peptides according to one of the claims 1 to 8, characterized in that the charge -stabilized leaving group is a compound of the formula

DIAGRAM

wherein applies:

A=O, S

and whereby R1 R2, R3, R4 and R5 may be independent of one another:

$-NO_2$, -CN, -F, -Cl, -Br, -I, $-CH_2Cl$, $-SO_3H$, -H, $-NH_3^+$, $-NL_3^+$, -C(=O)L, -C(=O)Het, $-O^-$, $-NL_2$, $-NH_2$, -OL, -OH, -NHC(=O)L, -OC(=O)L, -SL, $-CO_2^-$, -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein

L= -alkyl, -alkenyl, -cycloalkyl, -cycloalkenyl, -heteroalkyl, -heterocycloalkyl, -aryl, -heteroaryl,

wherein -alkyl stands for a group with 1 to 20 carbon atoms and -alkenyl for a monounsaturated or

polyunsaturated group with 2 to 20 carbon atoms and -alkyl or -alkenyl may be linear or branched; -cycloalkyl and -cycloalkenyl stand for a group with 3 to 20 carbon atoms, the heterocyclic groups stand for a residue with 1 to 20 carbon atoms wherein up to 5 carbon atoms may be substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus; aryl stands for an aromatic residue with 5 to 20 carbon atoms and heteroaryl stands for a corresponding aromatic residue in which up to 5 carbon atoms are substituted by heteroatoms chosen from the group nitrogen, oxygen, sulfur, phosphorus.

10. Method for the production of a substrate and subsequent reaction of this substrate with peptide cyclases into a cyclic peptide, wherein the substrates are linear peptides, characterized in that the following steps are carried out one after the other:

- Adding a reagent activating the C -terminus of the peptide acid, a coupling additive and a charge -stabilized leaving group to the free peptide acid in a solvent
- Stirring at room temperature,
- Addition of a base and further stirring at room temperature,

- Filtration,
- Removal of the solvent,
- Deprotection of the peptide,
- Addition of a peptide cyclase,
- Purification of the cyclic peptide obtained.

11. Method for the production of a substrate and subsequent reaction of this substrate with peptide cyclases into a cyclic peptide according to claim 10, characterized in that the acyl group of the C -terminal amino acid of the linear peptide is bound to one of the leaving groups according to one of the claims 5 to 9.

12. Method for the production of a substrate and subsequent reaction of this substrate with peptide cyclases into a cyclic peptide according to claim 11, characterized in that the leaving group possesses a pK_a value less than or equal to 10, preferably less than or equal to 8.

13. Method for the production of a substrate and subsequent reaction of this substrate with peptide cyclases into a cyclic peptide according to one of the claims 10 to 12, characterized in that DCC, DCI, PyClop, HBTU, HATU, HOSu, TBTU, T3P, BopCl or 3 -Cl -1

-pyridinium iodide are used as an activation reagent for the free C -terminus or a side chain carboxylic acid.

14. Method for the production of a substrate and subsequent reaction of this substrate with peptide cyclases into a cyclic peptide according to one of the claims 10 to 13, characterized in that HOBt, HOAt or HONB are used as a coupling additive.

15. Use of cyclic peptides according to one of claims 1 to 14 for the production of a pharmaceutical for the therapy, diagnosis and proplyaxis of diseases in which bacterial infections occur.

16. Use of cyclic peptides according to one of the claims 1 to 15 to produce a kit for the production of cyclic peptides.